Trends in Cell Biology

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Review

Opportunities and challenges for deep learning in cell dynamics research

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The growth of artificial intelligence (AI) has led to an increase in the adoption of computer vision and deep learning (DL) techniques for the evaluation of microscopy images and movies. This adoption has not only addressed hurdles in quantitative analysis of dynamic cell biological processes but has also started to support advances in drug development, precision medicine, and genome–phenome mapping. We survey existing AI-based techniques and tools, as well as open-source datasets, with a specific focus on the computational tasks of segmentation, classification, and tracking of cellular and subcellular structures and dynamics. We summarise long-standing challenges in microscopy video analysis from a computational perspective and review emerging research frontiers and innovative applications for DL-guided automation in cell dynamics research.

Highlights

Artificial intelligence (AI)-guided methods are transforming the speed and scale with which image segmentation and classification tasks can be managed in cell biology.

Deep learning (DL)-guided tools to segment and classify a variety of cells and subcellular structures are being rapidly developed, opening the need for standards and repositories.

Despite DL-guided advances in still-image analysis, tracking objects in microscopy movies remains an area of open development owing to spatial and temporal discontinuities.

DL methods offer new opportunities to significantly expand genotype–phenotype maps, genetic variant analysis, and drug development and discovery.

Automated analysis of cell dynamics

Advances in microscopy have influenced a range of areas in cell biology and biomedical research. Microscopy advances supported by automated or semi-automated image analysis are being transformed by DL approaches. DL methods for the analysis and restoration of microscopy image datasets have been reviewed recently [1,2], but there is no comprehensive survey of the status of AI methods for tracking or predicting the trajectories of dynamic structures in microscopy movies. Time-lapse movies of dynamic cell biological processes are particularly a unique case because of the temporal discontinuity in image acquisition which is being offset through high-speed and volumetric imaging [3–5]. Machine learning or deep learning (ML/DL) methodologies that demonstrate superior performance in most image analysis tasks are yet to be adapted for movie analysis tasks.

Implementing DL approaches involves **data annotation** (see Glossary), denoising, selection and training of a chosen **neural network**, evaluating and optimising the DL model, and assessment of outcomes – all dependent on specific imaging and analysis tasks. For a practical guide on how to build DL models for image analysis, we refer readers to a review focusing on bioimage analysis workflows [6].

In this review we present an in-depth survey of current AI-based microscopy image and movie analysis from the perspective of three key computational tasks: object **segmentation**, classification, and tracking. We contrast conventional image analysis approaches against DL techniques (neural network architectures) that have been successfully used in cell biology. To benefit future DL tool development, we collate a list of existing open-source datasets. Throughout we discuss accurate and efficient methods of data preparation for use in DL applications. Finally, we highlight key challenges and limitations of current DL applications in analysing dynamic cell biology movies, and identify opportunities for future DL-guided research developments.

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AI-guided advances in image analysis

We open with a brief illustration of successes in microscopy image analysis that have been enabled by ML/DL methods, and list how these can set new trends in cell biology. First, we can analyse large image datasets in a context-free and efficient way. This is ideal for large time-lapse videos or genome-wide imaging screens. Second, we are automating computational tasks, such as, image segmentation, classification, tracking, and transformation which support high-fidelity spatiotemporal studies of cellular processes. Third, we are able to recognise complex structures by recovering hidden patterns among known morphological features for hypothesis building and better data interpretation. Fourth, we can better manage noise and variation. In particular, handling morphological and intensity variations can bolster data reproducibility and reduce the chances of human biases or errors.

Table 1 lists the most widely used DL techniques for microscopy image analysis. Apart from these well-established techniques, a reusable and adaptable image segmentation architecture utilising a **zero-shot learning** approach, the Segment Anything Model (SAM) has been recently proposed by Meta AI [7]. Its performance appears to be competitive with or even superior to earlier fully supervised trained models and has been applied in medical imaging [8] and digital pathology [9]. SAM is unexplored for cellular or subcellular segmentation tasks, but it encounters challenges with intricate subcellular structures [10]. Evidently, SAM has the ability to simplify segmentation, but it has not yet been tested in densely packed microscopy images. For instance, electron microscopy (EM) images displaying crowded organelles may pose challenges to achieving accurate segmentation without trained datasets of individual organelles.

AI-guided methods outperform conventional image analysis tools

DL neural networks are more effective than traditional computer vision techniques. They learn from large-scale datasets and have the capacity to extract high-level features without heavy reliance on domain knowledge for feature extraction [11]. Although many DL tools have focused on segmenting nuclei and whole cells labelled with fluorescent markers, some specialised DL tools have been developed to segment distinct organelles such as the Golgi apparatus, mitochondria, and endoplasmic reticulum from EM data (Table 2). However, DL tools that can both segment and track dynamic subcellular structures in time-lapse fluorescent movies are currently limited. Mitochondria [12], microtubule ends [13], and mitotic spindles [14] are among the few dynamically changing structures for which automated analysis tools are available, but DL has only been used in the last case. Popular DL-based tools include U-Net [15,16], StarDist [17,18], and Cellpose [19,20]. Because most DL-based solutions are data-driven, there are no standards to inform biologists which model is most suitable for their own dataset and specific computational tasks. As a result, most people veer towards integrated platforms such as Fiji (through plugins) [21], CellProfiler [22], QuPath [23], ZEISS arivis Cloud (formerly APEER) [24], and ZeroCostDL4Mic [25]. We discuss below the application of DL to cellular image and movie analysis via segmentation, classification, and tracking, and contrast it against conventional non-DL methods.

Table 1. Deep learning (DL) techniques for cell biology

DL techniques	Applications in microscopy data analysis
Convolutional neural networks [88]	Segmentation [118], classification [119–121], tracking [76]
Recurrent neural networks [56]	Cell tracking [76], segmentation [122], cell-cycle analysis [123,124]
U-Net [15]	Segmentation [15,16,82,125], denoising [126], feature extraction [127]
Generative adversarial networks [128]	Image denoising [129,130], data augmentation [81], virtual staining of biological samples [131]
Graph neural networks [132]	Cell tracking [1]

Glossary

Data annotation: the process of adding attributes to training data and labelling them such that a DL model can learn what predictions it is expected to make.
Edge-based segmentation: a conventional segmentation approach that aims to first detect the contours of the specific object and then fill in the contours for segmentation.
Instance classification: usually consists of object detection, localising their position within the image, and classifying them into predefined categories.
Long short-term memory (LSTM): a type of recurrent neural network (RNN) architecture that was designed to overcome the problem of vanishing and exploding gradients faced by standard RNNs. LSTM is suited to tasks involving sequences with long-term dependencies, such as time-series prediction, natural language processing, and speech recognition.
Neural network: a densely interconnected group of nodes. Each node connects to several nodes in the layer beneath it, from which it receives data (e.g., training data in the last layer), and several nodes in the layer above it, to which it outputs data. Incoming connections are assigned weights. Active nodes multiply their respective weights and pass each forward if it exceeds a threshold. Training involves adjusting weights and thresholds are adjusted to produce similar outputs for data with the same labels. Examples include feedforward neural networks (FNNs), convolutional neural networks (CNNs), and recurrent neural networks (RNNs).
Segmentation: the process of dividing an image into multiple regions or segments where each corresponds to a specific object or area of interest.
Single-shot detector (SSD): an object-detection method that simultaneously predicts multiple bounding boxes and class scores for each box in a single pass. Unlike YOLO, SSD operates on multiple feature maps with different resolutions to handle objects of various sizes.
Thresholding segmentation: a conventional segmentation method that chooses a threshold based on the intensity histogram for segmenting an object.
You only look once (YOLO): an object detection method with the key idea of applying a single neural network to the

Segmentation

Two types of image segmentation, semantic and instance, serve different purposes. Semantic segmentation aims to classify individual pixels within an image into specific classes (Figure 1A, top). It groups objects of a class together, but lacks the ability to differentiate between individual objects such as overlapping nuclei. However, this approach effectively separates membrane outlines from intra- or extracellular space. Instance segmentation differentiates objects of the same class (Figure 1A, bottom). For example, Cellpose [19,20] and SAM [7] can separate overlapping nuclear objects, and treat them as separate entities, thus allowing the differentiation of individual nuclei even when they overlap. Recently, a new type of learning model called panoptic segmentation has been introduced which integrates instance and semantic segmentation. It identifies individual objects and labels each pixel with what it represents (a semantic category) [26].

Conventional segmentation methods include **thresholding segmentation**, edge-based algorithms, and region-based segmentation [27]. **Edge-based segmentation** methods such as Canny and Sobel edge-detectors followed by contour filling [27] perform better than thresholding, but can produce imperfect contours. Region-based segmentation, watershed segmentation in particular, is widely used in cell biology [27]. Conventional segmentation methods are often used for automated annotation of large datasets, followed by manual correction to save annotation time [14].

DL methods not only surpass conventional techniques in the segmentation of subcellular structures in microscopy images but also exhibit a remarkable generalisation capacity, and accommodate diverse imaging conditions, fluorescent markers or proteins, and cell types [14,28,29]. This has led to the creation of several freely available tools that provide pretrained models for biologists to segment and subsequently analyse microscopy datasets in a quantitative manner (Table 2).

Classification

Classification refers to assigning text labels to images and is frequently used in cell biology and digital pathology. **Instance classification** focuses on recognising and categorising individual objects within an image rather than classifying the image as a whole. DL techniques are used to identify and classify individual cells, nuclei or subcellular structures (Figure 1B), as well as to provide quantitative information about cell populations and their distribution [30,31]. Cell type and subcellular structure identification are other applications of instance classification, and have allowed robust quantitative studies of cell function [32], cell interaction [33], phenotype ('yes' or 'no' prediction) [34], and spatial patterns and protein localisation in fluorescence images [35,36]. Classification has also been used for large-scale phenotypic profiling of small molecules by analysing cellular responses to drug treatments at the single-cell level [37] to evaluate drug efficacy, mechanism of action, and potential side effects.

Manual annotations by cell biology experts are robust but time-consuming and expensive. To offset this cost, active learning [38] has been proposed. Active learning is a powerful human-in-the-loop process in DL. It involves annotating manually a subset of (not all) relevant objects in images, training with this subset, and generating initial segmentation and classification masks for all instances including unannotated ones [39]. Then, the autogenerated initial segmentation and classification can be reviewed and manually corrected, which then serve as annotations of the next training iteration, thus making the human-in-the-loop process a cost-efficient approach [14,19].

Unlike DL methods used for image classification, traditional ML-based classifiers are humanly interpretable, which is important for failure analysis and model improvement [40]. Although the DL framework has higher recognition accuracy on large sample datasets, the traditional ML approach (e.g., support vector machine, SVM) is thought to be a better solution for small datasets

full image, which then divides the image into regions and predicts bounding boxes and probabilities for each region. The high speed and accuracy of YOLO make it suitable for real-time tracking of objects.

Zero-shot learning: a remarkable machine learning/deep learning (ML/DL) method which refers to recognising new unseen objects; it can therefore be applied to new image distributions and tasks.

Table 2. Deep learning-based tools for subcellular organelle segmentation

Tool (Refs)	Subcellular structures[a]	DL architecture	Dynamics tracking	Strengths in user experience	Source
U-Net[b] [15,16]	Fluorescent and label-free cell membrane, fluorescent nuclei, and EM neurites	CNN	No	Documentation (application), tutorials (Jupyter notebook)	https://lmb.informatik.uni-freiburg.de/people/ronneber/u-net/
Cellpose[b] [19,20]	Fluorescent cell membrane and nuclei	U-Net	No	Documentation (installation and application), tutorials (Jupyter notebook), integrated through the ZEISS arivis Cloud (formerly APEER) [24]	https://github.com/mouseland/cellpose
Stardist (3D)[b] [17,18]	Fluorescent and H&E-stained nuclei	U-Net	No	Documentation (installation and application), tutorials (Jupyter notebook), integrated plugin (ImageJ/Fiji [21], QuPath [23])	https://github.com/stardist/stardist
ASEM [133]	EM Golgi apparatus, mitochondria, nuclear pore complexes, caveolae, endoplasmic reticulum, clathrin-coated pits, vesicles	3D U-Net	No	Documentation (installation and application)	https://github.com/kirchhausenlab/incasem
MitoSegNet[b] [29]	Fluorescent mitochondria	U-Net	No	Documentation (application), GUI (multiple operation systems)	https://github.com/MitoSegNet/MitoS-segmentation-tool
SpinX[a] [14]	Fluorescent mitotic spindle, label-free cell cortex	Mask R-CNN	Yes	Documentation (application), integrated through the ZEISS arivis Cloud (formerly APEER) [24]	https://github.com/Draviam-lab/spinx_local
Multicut [134]	EM neural membrane	U-Net	No	Documentation (installation), tutorials (Jupyter notebook)	https://github.com/ilastik/nature_methods_multicut_pipeline
nucleAIzer [135]	Fluorescent and H&E-stained nuclei	Mask R-CNN, U-Net	No	Documentation (installation and application), tutorials (shell scripts)	https://github.com/spreka/biomagdsb
DenoiSeg [136]	Fluorescent cell membrane and nuclei	U-Net	No	Documentation (installation), tutorials (Jupyter notebook)	https://github.com/juglab/DenoiSeg
InstantDL [137]	Fluorescent, H&E-stained, and label-free nuclei	U-Net, Mask R-CNN	No	Documentation (installation and applications), dockerised	https://github.com/marrlab/InstantDL
DeepCell [73,138]	Fluorescent nuclei and cell membrane	ResNet50	Yes	Documentation (application), tutorials (script), dockerised	https://github.com/vanvalenlab/deepcell-applications
SplineDist [139]	Fluorescent and H&E-stained nuclei	SartDist (U-Net)	No	Tutorials (Jupyter notebook)	https://github.com/uhlmanngroup/splinedist
CDeep3M [140]	XRM, ET, fluorescent and SBEM nuclei, SBEM synaptic vesicles, mitochondria and membranes	DeepEM3D-Net (dense CNN)	No	Documentation (installation), dockerised, implemented through Amazon Web Services	https://github.com/CRBS/cdeep3m
CellSeg [141]	Fluorescent nuclei and cell membrane	Mask R-CNN	No	Tutorials (Jupyter notebook)	https://github.com/michaellee1/CellSeg
EmbedSeg [142]	Fluorescent cell membrane and nuclei	Branched ERF-Net (3D)	No	Documentation (installation), datasets provided for reproducibility	https://github.com/juglab/EmbedSeg

[a]Abbreviations: EM, electron microscopy; ET, electron tomography; H&E, haematoxylin–eosin; SBEM, serial block-face electron microscopy; XRM, X-ray microscopy.
[b]Tools with comprehensive documentation and tutorials that can be accessed independently of the source code.

[41]. Hybrid approaches that combine ML and DL techniques are therefore being used for high accuracy and precision for cell type classification problems [42] as a step towards explainable AI.

Tracking

Tracking is the process of identifying and linking the movement of specific objects over time in a series of time-lapse images or a movie. Tracking methods in cell biology are primarily DL-

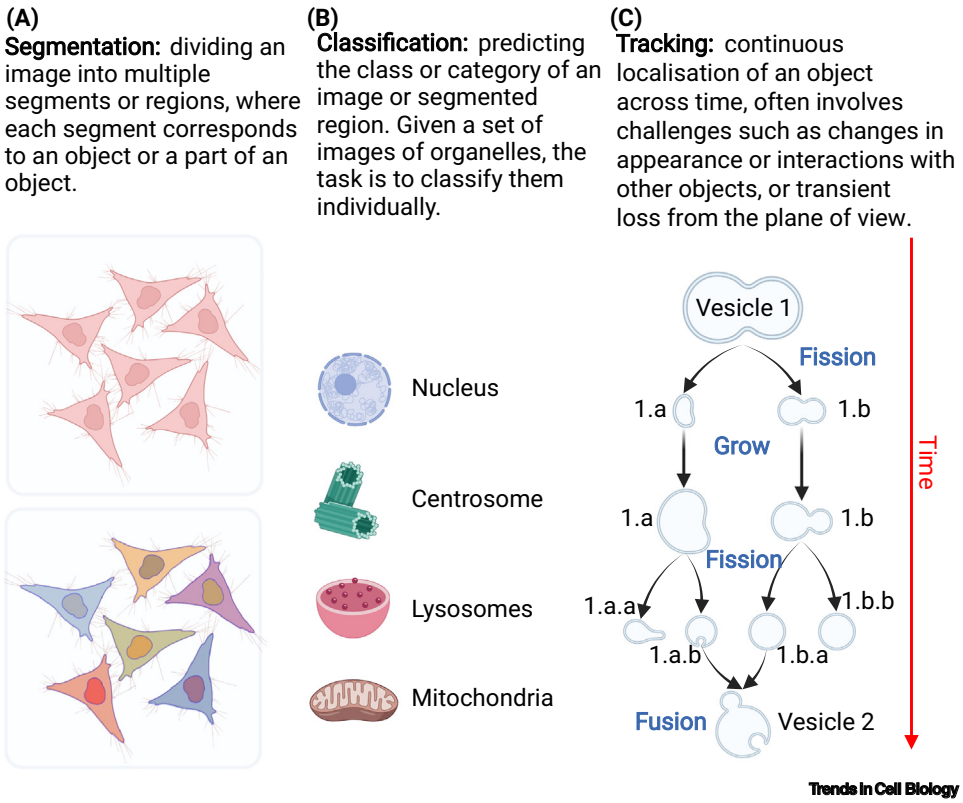

Figure 1. Deep learning (DL)-guided methods to analyse still images and time-lapse movies. (A) Image segmentation tasks, semantic and instance, that serve different purposes. Semantic (top) treats multiple objects within a single category (cell or nucleus) as one entity, whereas instance (bottom) identifies individual objects within a category. (B) Image classification tasks to categorise objects (cells or nuclei) within an image. This task requires a predefined set of classes (e.g., organelle names). The output is a single label from the set of classes. (C) Object tracking where segmented and classified objects are monitored through time to follow changes in object morphology or intensity. The cartoon shows an example of vesicle tracking through time. Vesicle numbers indicate the complex nomenclature that is necessary to manage dynamic changes in morphology and interactions during vesicle fission, fusion, or growth events. Figure created with BioRender (https://biorender.com/).

independent, unlike real-world scenarios such as autonomous driving where DL-based tracking is being widely used [43–47]. From a computational perspective, the task of tracking consists of detection-based tracking (DBT) and detection-free tracking (DFT) [48]. DBT, also commonly referred to as tracking-by-detection, usually consists of two main steps: detection of the objects of interest, and linking their positions and properties across consecutive frames. On the other hand, DFT requires manual initialisation of a fixed number of objects in the first frame and then localising (location-identification) these objects in the subsequent frames. DBT is widely used compared to DFT because objects can be newly discovered or transiently lost through time in most scenarios, and DFT cannot deal with such cases [48].

In many tracking studies, DL is used in the detection step as in the R-CNN series [49–51], **you only look once (YOLO)** [52–54] and **single-shot detector (SSD)** [55]. DL can also be used for trajectory or motion prediction to support tracking. Most DL-based trajectory predictions use the **long short-term memory (LSTM)** technique [56] which has extensively progressed by predicting the coordinates of selected objects in the upcoming time frame [57–60]. Some studies have taken advantage of convolutional feature extraction [43] for predicting trajectories. Currently [44–47] the top application scenarios of DL-based tracking are pedestrian detection and

autonomous vehicles – augmented reality (AR) and virtual reality (VR) [61,62]. Similar DL-based tracking could be brought to cell biology to advance multiscale system studies where subcellular-, cellular-, and tissue-level changes are simultaneously modulated and measured.

Typical examples for tracking in cell biology applications include single-cell tracking [63], multi-cell tracking during collective cell migration [64], and particle or organelle tracking within cells [65,66]. Tracking is challenging from both computational and biological perspectives for many reasons. First, objects can move from area to area; each instance should therefore be identified on a single-frame basis and these detections should be linked over time to avoid misconnections. Second, objects that are to be tracked can merge (mitochondria) or vesicles or split (cell division), and this presents a discontinuity challenge in their morphology, leading to misrecognition (Figure 1C). Third, there is a limitation in terms of the frame rate in time-lapse movies [67,68], and this makes tracking in general, and in 3D in particular, challenging because of time discontinuity. Misconnection and misrecognition challenges could be overcome at least in part by using DL methods for trajectory prediction, and live predictions can facilitate microscope-based physical tracking of objects.

Tracking subcellular structures and their changes through 3D space is a challenging but rewarding application because it can provide valuable insights into cell dynamics [69,70] and support systems-level modelling efforts to explore complex signalling and regulatory pathways [71,72]. For example, analysis of the patterns of cell movements following distinct molecular perturbations has helped to dissect the molecular principles that govern cellular migration [73–75]. Whole-cell tracking to monitor cell or nuclear size changes and the timing and duration of cell-cycle phases [14], or intracellular tracking to analyse the movement of intracellular organelles, vesicles, or proteins within a cell [66,76,77], have taken advantage of *a priori* knowledge of distinct features (structural or dynamic) which have been uniquely used to solve each individual tracking problem.

The challenges and opportunities

Challenges of AI-guided methods in cell dynamics studies

Lack of well-annotated datasets

DL-based approaches require large amounts of labelled (annotated) data. Ideally, high-quality cell biology data need to be annotated by experts, which is time-consuming. Although crowdsourcing can offer cost-effective solutions, annotation inconsistencies would require correction by experts [78]. Furthermore, variations in subcellular morphologies, staining protocols, and imaging quality can make the annotation challenging for non-experts. Many solutions are being developed to tackle this challenge [1], including active learning [79], transfer learning [79,80], and data augmentation techniques [81]. Augmentation strategies where an image is altered in scale or intensity provide additional samples without necessarily increasing the number of manually annotated samples [14]. Karabag and colleagues investigated the impact of the amount of training data and shape variability on U-net-based segmentation [82]. They suggest that data augmentation methodologies may not improve training if the acquired cell pairs are not representative of other cells. Therefore, thorough investigation of various augmentations is recommended. Despite the mentioned solutions, the shortage of high-quality labelled data remains a crucial limitation for AI-guided analysis of images and time-lapse movies. Only a limited number of open-source datasets are available, as listed chronologically in Table 3.

The quality of image datasets

DL models rely on extracting patterns and features from a dataset, making the quality of the annotated data crucial. Inconsistent ground truth yields incorrect analytical results, whereas biased data (highlighting some but not all phenotypes) can lead to incorrect patterns or inaccurate predictions. Noise intrinsic to microscopy can also increase the complexity of the model that is

Table 3. Open-source datasets for cell biology image and movie analysis tasks

Dataset (Refs)	Description	Source
Broad Bioimage Benchmark Collection [143]	Over 11 million images from 52 datasets for segmentation, phenotype classification, and image-based profiling tasks	https://bbbc.broadinstitute.org/image_sets
ISBI cell tracking challenge dataset collection [144]	Ten 2D image datasets and ten 3D time-lapse movie datasets of fluorescent counterstained nuclei or cells for segmentation and tracking tasks	http://www.celltrackingchallenge.net/
DeepCell dataset [73]	~75 000 single-cell annotations including live-cell movies of fluorescent nuclei (~10 000 single-cell movie trajectories over 30 frames) and static images of whole cells for segmentation tasks	https://github.com/vanvalenlab/deepcell-tf
Image data resource (IDR) [85]	Over 13 million images from 118 published studies	https://idr.openmicroscopy.org/
Human Protein Atlas [145]	Over 80 000 high-resolution confocal immunofluorescence images showing localisation patterns of thousands of proteins for a variety of human cell lines for segmentation tasks	http://www.proteinatlas.org
The Cell Image Library [146]	919 874 five-channel fields of morphologies of U2OS cells and populations representing 30 616 tested compounds	https://github.com/gigascience/paper-bray2017
Salmonella-infected HeLa cells [147]	93 300 multi-channel confocal fluorescence images	https://dataverse.harvard.edu/dataset.xhtml?persistentId=doi:10.7910/DVN/FYGHFO
JUMP cell painting datasets [148]	Images of osteosarcoma cells perturbed with CRISPR-mediated knockdowns and overexpression reagents and ~120 000 compounds	https://jump-cellpainting.broadinstitute.org/
NYSCF automated deep phenotyping dataset (ADPD) [149]	Cell painting dataset of 1.2 million images (48 TB)	https://nyscf.org/open-source/nyscf-adpd/
Poisson–Gaussian fluorescence microscopy denoising dataset [150]	Over 12 000 fluorescence microscopy images using confocal, two-photon and widefield microscopes	https://drive.google.com/drive/folders/1aygMzSDdoq63lqSk-ly8cMq0_owup8UM

necessary to accurately capture the underlying features. This may lead to overfitting, where the model becomes too complex and fails to generalise to new and unseen data. Noisy data can also lead to challenges for DL models, resulting in under- or over-segmentation of cells or misclassification of cell types [83], which could lead to incorrect tracking of cells in movies. Meiniel and colleagues present a comprehensive review of current techniques for denoising microscopy images, and they introduce a novel sparsity-based method for enhanced image clarity [84] which leverages the inherent sparsity in microscopy images and offers improved denoising performance compared to existing methods [84]. To manage the problem of high-quality image availability, the image data resource has been set up to allow easy image data access, storage, and dissemination [85]. Overall, it is essential to ensure that the datasets used for DL are of high quality, with solid ground truth and minimal noise, and are free from bias [86].

Model interpretability

The challenge of interpretability for DL models arises from the complex and black-box nature of these models [87]. DL models can automatically extract complex features and patterns from large amounts of data through multiple layers of neurons [88]. Although this makes such models powerful, in tasks such as image segmentation or classification, it also presents a challenge in understanding how the models arrived at their predictions or decisions. One way to address this challenge is to visualise and examine the activations of individual neurons or groups of neurons within the model [89]. This technique provides insights into the patterns that the model has used to form its decision. However, these visualisations may be difficult to interpret without a deep understanding of the model architecture and data domain ([90] for more information).

High cost in real-world scenarios

DL-based methods are often expensive due to two main factors. First, effective training of DL models requires a large amount of data which can be expensive to generate. Second, the training process can be computationally intensive, requiring high-performance computing resources such as hardware of graphics processing units (GPUs) and tensor processing units (TPUs). This infrastructure cost can dissuade the planning of imaging studies that are necessary to build the DL model [91]. DL model-building efforts supported by agencies/consortiums beyond individual researchers can help to meet upfront costs and maintain standards to make sure that the models are reusable [92].

The generalisability issue

Generalisability denotes the extent to which a DL model trained on a specific dataset might perform well on new data, especially when the new data have different features or patterns compared to the training data. To showcase generalisability, DL models are deployed on data acquired from a different cell type or microscope [30,31]. Efforts to reuse or generalise workflows are ongoing [75]. Generalisability issues arising due to sample variability or differences in image acquisition are being addressed through data augmentation, multi-task learning, swarm learning, or collaboration with domain specialists [93,94].

Opportunities for AI-guided methods in cell dynamics studies

With the advent of new AI-guided methods to identify, track, and analyse objects in time-lapse movie datasets (Table 3), we expect new opportunities for large-scale cell biological applications in drug discovery, drug repositioning, and phenome–genome interaction map-building efforts.

Drug discovery and repositioning

AI approaches in microscopy-based drug development or drug target identification primarily use still image datasets which are snapshots of dynamic processes [2,95]. Such still image-based drug screening efforts do not yet fully benefit from cellular and subcellular dynamics that can be visualised using high-speed live-imaging microscopes [3,96,97]. Incorporating dynamic changes through time can address challenges posed by cellular heterogeneity, cell cycle stages, cell fate dissimilarities, variations in protein expression and in cellular or subcellular dimensions, and inter/intracellular signalling [98]. In addition to taking advantage of cell dynamics principles, AI-guided methods for movie datasets can accelerate several steps of drug discovery including cell toxicity assays [99], cell cycle profiling, and morphology analysis [98,100]. Increasing single-cell movie datasets together with the development of DL model standards can integrate image-omics with other omic datasets that capture dynamic information and have accelerated drug repositioning studies [101,102]. Investing in collaborative efforts to compile microscopy datasets can fuel the development of robust AI-guided methods. This in turn will unlock research

and engineering opportunities, thereby facilitating a cyclical learning process to uncover unexplored cellular transition states in frontier biology and drug discovery studies.

Genome–phenome mapping

Genetic interaction maps built using cell biological approaches are transforming our understanding of several biological processes [103], but their influence is limited to the specific model system or experimental setup. We are only beginning to reliably link datasets from different cell types, fluorescent markers, or imaging systems [104,105]. AI-guided image analysis methods are well positioned to extract information across image and video datasets, and across different databases, in an unbiased form because they can be trained to search for patterns (e.g., nuclear atypia such as multinucleated, misshapen, and binucleated structures [106] could be gathered across hundreds of cell lines or drug treatments). Currently, high-throughput genome–phenome mapping image datasets of various cell types and models are deposited in a disconnected fashion because there is not much incentive to unify them. AI-guided methods may offer the possibility and the value of developing universal standards for collating data, in addition to existing global efforts to name and store large movie datasets [107,108].

Precision medicine

Genetic variant interpretation and classification using high-throughput cell biological methods is still a nascent field. Germline variant guidelines are well established [109] and somatic variant guidelines are being established [110]. In both cases we expect single-cell imaging, the associated image dataset, and image analysis methods to play a crucial role in stratifying variant pathogenicity. To build stratification methods that are scalable, generalisable, and interrogative (crosscheck), DL models could be trained to detect and classify phenotype changes and hidden patterns. Swarm learning has been proposed for decentralised and confidential X-ray image analysis [111] and digital pathology [112], and could be extended to cell biological images and live-cell movies. As AI methods become incorporated within the clinical prognosis framework [113,114], we predict there will be a growing demand for robust models for evaluating the clinical actionability of molecular targets in cancer therapies, genetic rare diseases, and infectious diseases.

Concluding remarks

The impact of DL methods in the analysis of large-scale and complex microscopy data has been significant. DL techniques have already revolutionised still-image analysis and are now beginning to transform time-lapse movie analysis through state-of-the-art performance in a wide range of applications, such as object detection and tracking, segmentation, and unsupervised clustering and classification. DL methods used to segment and classify cells are beginning to detect novel anomalies in 3D structures [115] or time-series data [116], identify distinctive transient cellular transitions [100], and reveal complex behaviours and movement patterns [14,117] which were previously unrecognised.

Automated and data-driven workflows together with cloud-based large-scale solutions have significantly improved the speed, efficiency, and accuracy of DL-guided image analysis tasks, while also increasing the ease with which biologists can implement and share AI tools. Overall, the use of DL methods in microscopy has enabled researchers to extract valuable information, some that is not obvious to the eye, from huge volumes of image data and has opened new opportunities in medical diagnosis and clinical translation.

It is important to recognise that DL methods rely on abundant, robustly annotated data and careful parameter-tuning. Assessing their reliability and interpretability can be challenging [86], which can restrict their applications in some domains (see Outstanding questions). The establishment of

Outstanding questions

Despite the rapid growth of DL-guided methods for microscopy image analysis, very few tools have been developed to be reusable and generalisable. The infrastructure costs associated with DL tool development are significant. Can scaling up of shared online spaces for model training and the adoption of universal data standards further accelerate the development of reusable DL models?

DL-guided tracking algorithms built specifically for cell biology can revolutionise long-term live imaging by enabling simultaneous tracking of rapidly moving objects. How can algorithms previously developed for tracking objects in autonomous driving be adapted to track dynamic cellular structures experiencing morphological changes?

Larger datasets of microscopy images and movies can support the development of new algorithms. How can annotated images and trained models be fairly reused to promote the storage of datasets in open-source image archives?

Despite the impressive scale and speed of AI-guided image analysis methods, their lack of transparency and interpretability has obscured connections and relationships within cell fates or phenotypes. What design efforts should be considered when building explainable AI methods for cell biology such that trustworthy biomedical and clinical applications can thrive?

universally accepted standards and frameworks to store and share human-annotated image datasets, DL models, and post-processing pipelines are complex challenges [91] that necessitate attention through international collaborations and consortia.

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Author contributions

B.C. drafted the manuscript together with V.M.D. and C.E., and edited sections using comments from H.Y. B.C. contributed to Figure 1 with support from V.M.D. and to Tables 1–3, H.Y. contributed to Table 1, and C.E. generated Table 2 with support from B.C. and V.M.D.

Declaration of interests

The authors declare no conflicts of interest.

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